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**SKIN AND GUT SPICULES IN
DISCODORIS ATROMACULATA (BERGH, 1880)
(MOLLUSCA: NUDIBRANCHIA) (**)**

KEY WORDS: Nudibranchs, Doridaceans, calcareous spicules

Summary

The presence of skin spicules inside the notum, the foot or in any other organs of numerous doridaceans is well known, and generally considered a defensive mechanism to diminish the nudibranch attractiveness as food.

About 80 specimens of *Discodoris atromaculata* (Bergh, 1880) (Nudibranchia: Doridina) were collected monthly for a year along the Portofino Promontory (Ligurian Sea), in order to clarify some aspects on the calcareous spicules morphology and the hypothetical use of the siliceous ones coming from diet on the sponge *Petrosia ficiformis*. The specimens' average foot length is 25.4 mm and their average wet weight is 3.2 g. In either morphometric bulk of data (total length, foot length, total width and wet weight) no significative differences can be outlined during the year. It probably occurs because this species shows a wide reproductive season (from April to October) and its life history suggests a biennial cycle.

The *D. atromaculata* notum shows blunt tubercles of various sizes supported by densely packed calcareous spicules covered by the epithelium, others lie in the inter-tubercles spaces. Other spicules are present in foot, gills and rhinophores, more irregularly arranged. A lot of these spicules is slender and spiny, those present in gills and rhinophores are sometimes curved or show a central bulge. Small sphaeroid spicules (diameter 20-80 μm) are always present. The slender spicules show a considerable intra- and interindividual variability that is not related to individual size or season. The average size of the notal slender spicules is 332 μm x 16 μm , while the pedal ones is 260 μm x 16 μm , and this difference is statistically significative ($p < 0.01$: t test and Kolmogorov-Smirnov). Consequently, the spicule length in *D. atromaculata* seems to be influenced by the anatomical localization and its development can be considered in terms of changes in gene regulation caused by the kind of tissue in which it occurs.

The percent value of calcium carbonate is always higher in the notum than the foot ($p < 0.01$), and this quantity does not change during the year, in either of these organs. On the contrary, a slightly negative relationship with the individual size can be underlined mainly in the notum, and this unidirectional percent decrease suggests a natural slowing down of the biomineralization related to the age.

The strong monophagy of *D. atromaculata* on *P. ficiformis* is confirmed by the gut contents analysis, but it does not appear absolute. In fact, one specimen, 25 mm long, with the gut full of spicules of the sponge *Reniera fulva* was collected on this sponge which showed evident signs of predation.

D. atromaculata does not suspend feeding activities during the year: monthly, specimens with the gut full of siliceous spicules were recorded, but the nudibranch never uses these alloctone spicules to firm its notum.

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Riassunto

La presenza di spicole calcaree nel notum, nel piede ed in altri organi di numerosi dorida-cei (Gastropoda: Nudibranchia) è ben nota e generalmente queste strutture vengono considerate elementi difensivi, atti a diminuire l'interesse alimentare della specie nei confronti di un eventuale predatore. Grazie allo studio condotto su oltre 80 individui di *Discodoris atromaculata* (Bergh, 1880) (Nudibranchia: Doridina), raccolti mensilmente per un anno lungo il Promontorio di Portofino (Mar Ligure), è stato possibile chiarire alcuni aspetti della morfologia di queste spicole ed escludere l'utilizzazione di spicole silicee, provenienti dalla dieta, da parte del nudibranco.

La lunghezza media del piede degli esemplari esaminati è di 25.4 mm, con un peso umido medio di 3.2 g. Analizzando il complesso dei dati morfometrici presi in considerazione (lunghezza totale, lunghezza del piede, larghezza, peso umido) non è stato possibile riscontrare differenze significative nelle diverse stagioni dell'anno. Ciò è dovuto al fatto che la specie presenta un ampio periodo riproduttivo (da aprile ad ottobre) con un ciclo probabilmente biennale.

I tubercoli del notum in *D. atromaculata* sono costituiti da strutture subconiche costituite da spicole calcaree coperte dall'epitelio. Gli interspazi tra i tubercoli sono riempiti da altre spicole. La maggior parte delle spicole calcaree sono fusiformi e spinose, mentre quelle presenti nelle branchie e nei rinofori possono talvolta essere più irregolari, con un rigonfiamento centrale. Piccole spicole sferoidali, del diametro di 20-80 μm sono sempre presenti. Le spicole fusiformi presentano un'alta variabilità inter- ed intraindividuale che non sembra essere correlata nè con la stagionalità nè con la taglia. La dimensione media delle spicole presenti nel notum è $332 \pm 63 \mu\text{m} \times 16 \pm 3 \mu\text{m}$, mentre quelle del piede sono statisticamente più piccole ($260 \pm 43 \mu\text{m} \times 16 \pm 3 \mu\text{m}$). La taglia delle spicole in *D. atromaculata* sembra dunque essere influenzata, più che da fattori ambientali esterni, dal tipo di ambiente cellulare o tissutale in cui vengono a formarsi.

Il valore percentuale di carbonato di calcio presente nel mantello è sempre statisticamente maggiore di quello misurato nel piede, e questa quantità non sembra variare nel corso dell'anno. Al contrario si nota un leggero decremento nel valore percentuale di carbonato di calcio con l'aumento della taglia dell'individuo, suggerendo una leggera, ma significativa riduzione dell'attività di biomineralizzazione legata all'età.

La monofagia di *D. atromaculata* per *Petrosia ficiformis*, pur confermata, non appare assoluta. L'analisi del contenuto stomacale di un individuo di 25 mm di lunghezza ha rivelato la presenza di notevoli quantità di spicole di *Reniera fulva*.

Introduction

The presence of calcareous spicules inside different body regions of numerous doridaceans is well known (KRESS, 1981; GARCIA et al., 1986). They appear in the notum of one mm long juvenile *Cadlina laevis* during the first 3 days after hatching (THOMPSON, 1967), and cannot be considered homologous of the shell, as they appear not only in the notum, but also in the foot, rhinophores and gills. Almost nothing appears to be known about spicules formation and turn-over in nudibranchs, but some information is available on the type of mineralization in few opisthobranchs (LOWENSTAM & WEINER, 1989).

During a field study carried out on some traits of the life history of *Discodoris atromaculata* (Bergh, 1880) (Nudibranchia: Doridina) in the Ligurian Sea, about 80 specimens were sampled along to Portofino Promontory, in order to clarify some aspects on the calcareous spicules morphology of this species, its diet, and the possible utilisation of the siliceous sponge spicules to firm the body.

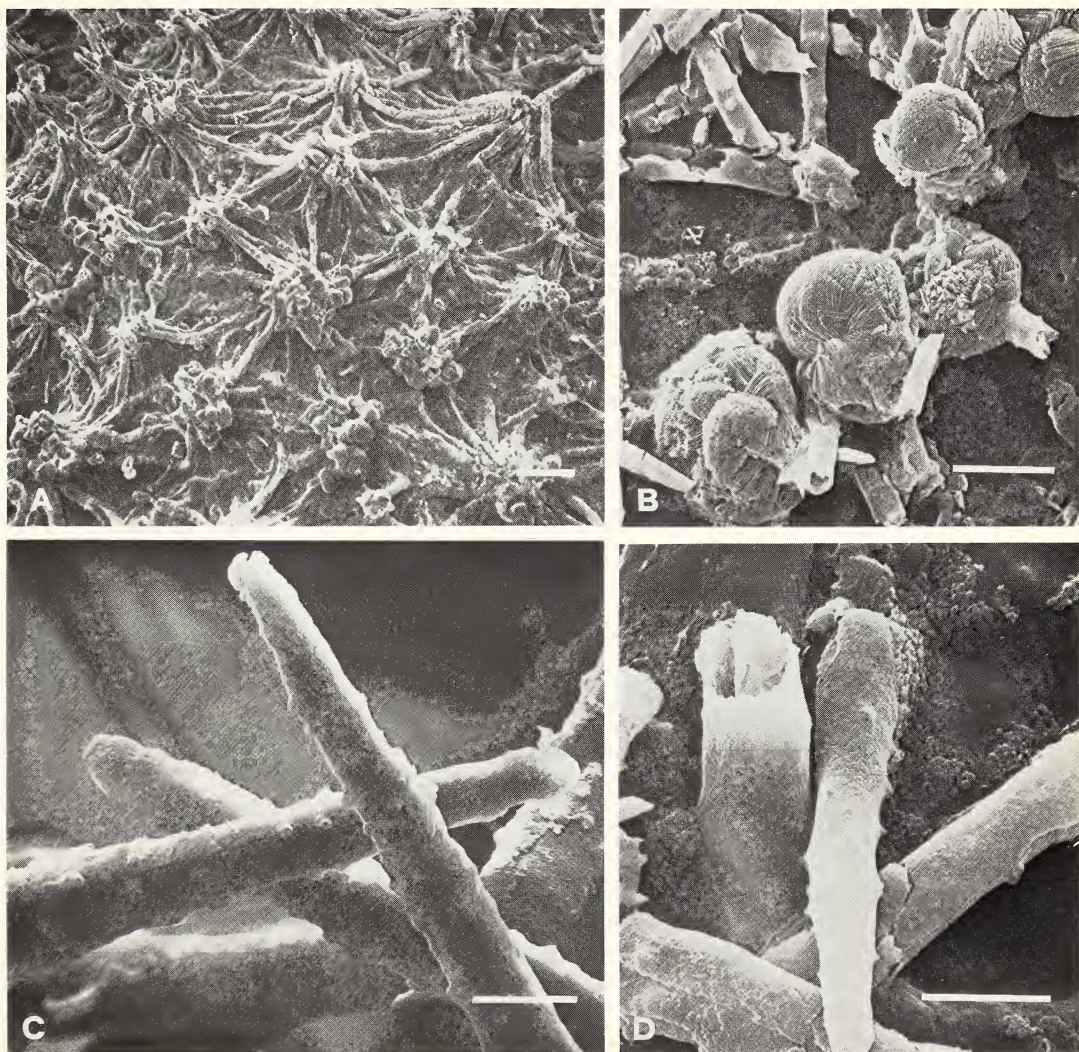


Fig. 1. *Discodoris atromaculata*: calcaeous spicules. a) notal subconical tubercles supported by densely packed calcaeous spicules. b) small calcium carbonate sphaerules present in the notum. c) slender spicules showing the spiny surface. d) Spicule showing the empty center. Bar length: a=100 μm ; b=40 μm ; c-d=20 μm .

Tab 1. *Discodoris atromaculata*: Descriptive statistics. Length in mm; weight in g.

	Whole Year			Warm Season			Cold Season		
	n	avg	st.dev.	n	avg	st.dev.	n	avg	st.dev
tot. length	82	39.29	19.36	52	42.59	20.55	30	33.57	15.83
tot. width	82	23.36	12.17	52	26.58	12.85	30	17.79	8.53
wet weight	82	3.18	3.70	52	3.38	3.80	30	2.84	3.56
foot length	82	25.40	11.60	52	26.67	11.64	30	23.20	11.39

Material and methods

82 specimens of *Discodoris atromaculata* were collected from March 1990 during monthly SCUBA dives along the Portofino Promontory (Ligurian Sea), for a year. All the specimens were found in the precoralligenous and coralligenous biocoenoses, at a depth of 15 to 30 m. The siliceous and calcareous spicules present in the digestive system, and in the notum, foot, rhinophores and gills were prepared on slides, respectively after dissolution of tissues by boiling nitric acid and H_2O_2 . For each slide, the length and width of 20 spicules were noted for a bulk of data of over 2000 spicules. The percent value of the calcium carbonate present in the notum and foot was determined in 1 cm² dry skin tissue, by weight difference after dissolution of the spicules in 2% hydrofluoric acid.

Results

Population data

Practically the entire studied population, both juveniles and adults, was collected on the common sponge *Petrosia ficiformis* Poiret, 1789, the usual prey of this nudibranch (SCHMEKEL & PORTMANN, 1982). The descriptive statistics of this population (total length, foot length, total width and wet weight) are summarized in Tab. 1. Data have been divided up into cold (from October to March) and warm season (from April to September) to clarify possible seasonal differences. The foot length varies from 9 mm to 48 mm (with an average value of 25.4 mm), while the wet weight from 0.01 g to 19.7 g (with an average value of 3.18 g). The various multiple range tests available in the SAS ANOVA package gave no statistical differences between the two seasonal groups. It could happen because in this species, which shows its reproductive season from April to October, the larval metamorphosis occurs during a wide period of the year and its life history suggests a biennial cycle.

Morphology of the calcareous spicules

The *Discodoris atromaculata* notum shows blunt tubercles of various sizes supported by densely packed calcareous spicules covered by the epithelium (Fig. 1a). No spicules protrude from it and they are arranged as indian teepees, while others lie in the inter-tubercles spaces. Other spicules are present in the foot, rhinophores and gills, more irregularly arranged.

Tab. 2. Monthly data regarding the length and width of the notal and pedal spicules (in μm).

	n° obs.	Notum		Foot	
		length	width	length	width
January	59	416 \pm 43	19 \pm 2	320 \pm 15	16 \pm 1
February	60	207 \pm 15	15 \pm 3	253 \pm 57	16 \pm 4
March	100	359 \pm 44	17 \pm 1	253 \pm 19	17 \pm 1
April	138	296 \pm 33	15 \pm 2	267 \pm 23	17 \pm 3
May	99	377 \pm 24	21 \pm 3	241 \pm 44	17 \pm 3
June	100	265 \pm 64	14 \pm 3	219 \pm 33	15 \pm 2
August	178	374 \pm 29	19 \pm 3	267 \pm 21	16 \pm 1
September	100	348 \pm 41	14 \pm 1	241 \pm 27	15 \pm 1
November	119	322 \pm 28	15 \pm 2	258 \pm 58	16 \pm 5
December	20	358 \pm 38	22 \pm 3	296 \pm 3	296 \pm 27

Tab. 3 Average length, width and their ratio in notal and pedal spicules.

	notum	foot
Length (μm)	332.6 \pm 63.1	260.1 \pm 42.8
Width (μm)	16.6 \pm 3.3	16.2 \pm 3.2
ratio W/L	0.05 \pm 0.01	0.06 \pm 0.01

Many calcium carbonate spherules (diameter 20-80 μm) are always present (Fig. 1b), but the main notal and pedal spicules are generally slender, lightly curved and often spiny (Fig. 1c). Those present in gills and rhinophores are sometimes more irregular and can show a central bulge. The spicules seems to be very fragile because have generally an empty center (Fig. 1d), but others show concentric layers inside. Also their walls have a layered structure. Some morphometric data concerning this kind of spicules are summarized in Tab. 2. The average length of the notal ones is 332.6 μm (\pm 63.1), its width is 16.6 μm (\pm 3.3). Those in the foot are 260.1 μm (\pm 42.8) long and 16.2 μm (\pm 3.2) wide. The difference between the lengths is statistically significative ($P < 0.01$: t test and Kolmogorov-Smirnov test) as well as the width/length ratio, which is consequently higher in the foot (Tab. 3).

The standard deviation high values inside both data suggest a strong inter- and intraindividual variability: strong irregular variations in the spicule length can be, in fact, observed during the year, but no morphological or morphometric changes can be related to the individual size or season.

The percent value of calcium carbonate present in the notum is always higher than in the foot, but this quantity does not change, in either of the structures, notably during the year (Tab. 4). On the contrary, a slightly negative relationship with the individual size can be underlined mainly in the notum (Fig. 2).

Siliceous spicules

The analysis of the gut contents showed the presence of the siliceous spicules of the sponge *Petrosia ficiformis* in almost all the specimens examined. Only one specimen, 25 mm long, collected on the sponge *Reniera fulva* which showed evident scars of predation, had its gut full of spicules of that sponge. The percentage of empty stomachs is constant during the whole year (about 60%) and, consequently, this species does not suspend feeding activities during the year. No siliceous spicules were never found in the notum and foot.

Discussion

The skin spicules, generally considered a defensive mechanism to diminish the nudibranch attractiveness as food to predators (THOMPSON, 1960; Ros, 1976), have an important role in determining the firmness, structure and architecture of the notum in *Discodoris atromaculata*. In this case no alloctone siliceous spicules occur. Contrary to HAEFELFINGER's (1961) observations, only a type of slender calcareous spicules is present in the Portofino population, even though sizes are statistically different between the notal and pedal spicules. A similar phenomenon occurs in the spicules of demospongiae (BAVESTRELLO et al., 1992) with a variation of the size of the same kind of spicules in different anatomical territories. This plasticity may be ascribed to the phenomenon of phenotypic modulation (SMITH-GILL, 1983) where the environment may modify in *continuo* the genetic expression. The decrease of calcium carbonate in the notum with the size suggests a natural slowing down of the biomineralization with age.

All this suggests that the process of biomineralization in *Discodoris atromaculata* is not directly influenced by seasonal changes (e.g. temperature), but it is better linked to the age of the specimen and the different anatomical localization.

The trophic relationship between the sponge *Petrosia ficiformis* and *D. atromaculata* is generally considered as one of the best examples of monophagy in nudibranchs (CHANGEUX & DELAMARE, 1955; HAEFELFINGER, 1961; VICENTE, 1967; SCHMEKEL & PORTMANN, 1982) and this close relationship was also demonstrated by biochemical results (CIMINO et al., 1980; CASTIELLO et al., 1980) and behavioural experiences (CASTIELLO et al., 1979). This fact can be confirmed, but it does not appear absolute. In fact, the feeding on *Reniera fulva* can create some doubts, because this last species is rarer than *Petrosia ficiformis* along the Portofino Promontory.

Discussion arised on the possibility of using sponge spicules to firm the nudibranch notum (VICENTE, 1967), but in *Discodoris atromaculata* they are confined always to the digestive system and consequently, this hypothesis would seem very unlikely.

Tab. 4 Monthly percentage values of calcium carbonate/cm² of dry notum and foot.

	n° obs.	notum %CaCO ₃	foot
January	3	58±2	43±11
February	2	68±2	56±5
March	9	65±8	49±10
April	10	65±9	54±13
May	11	69±7	50±10
June	4	61±8	46±6
August	8	61±9	51±8
September	5	68±6	47±4
November	8	68±8	41±12
December	3	68±7	58±8

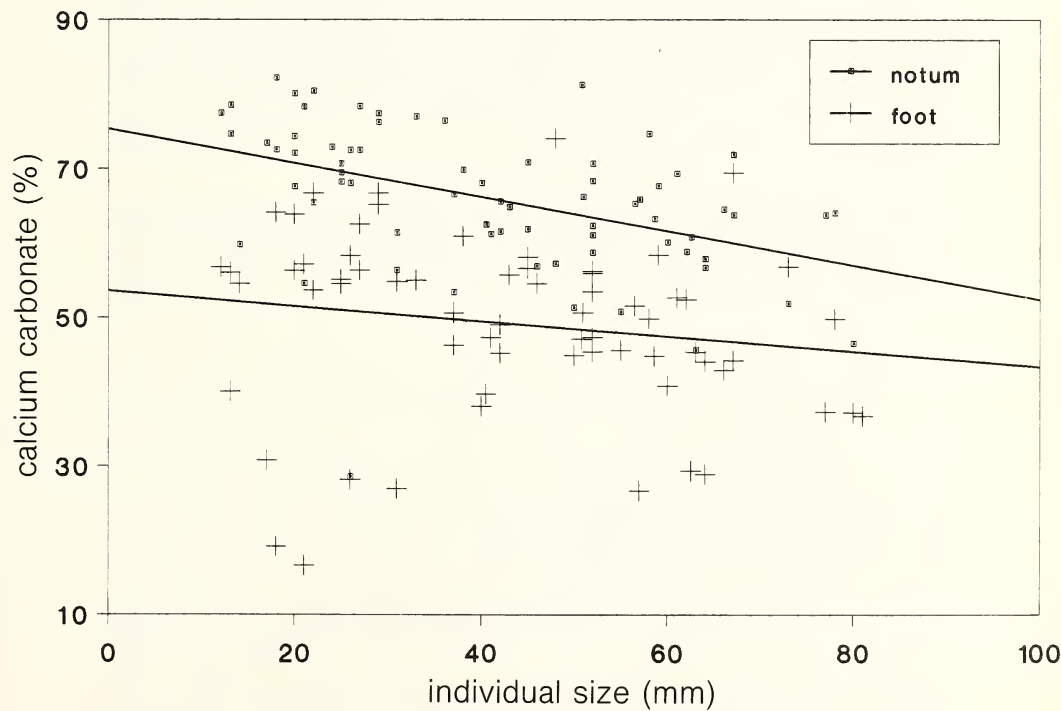


Fig. 2. *Discodoris atromaculata*: Individual size vs per cent calcium carbonate (d.w.) in the notum and foot of 63 specimens.

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